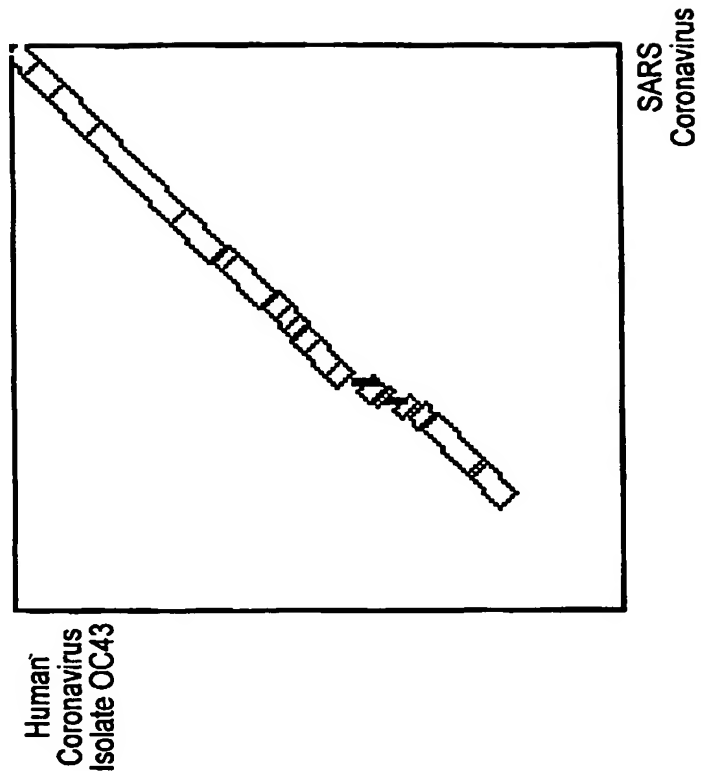
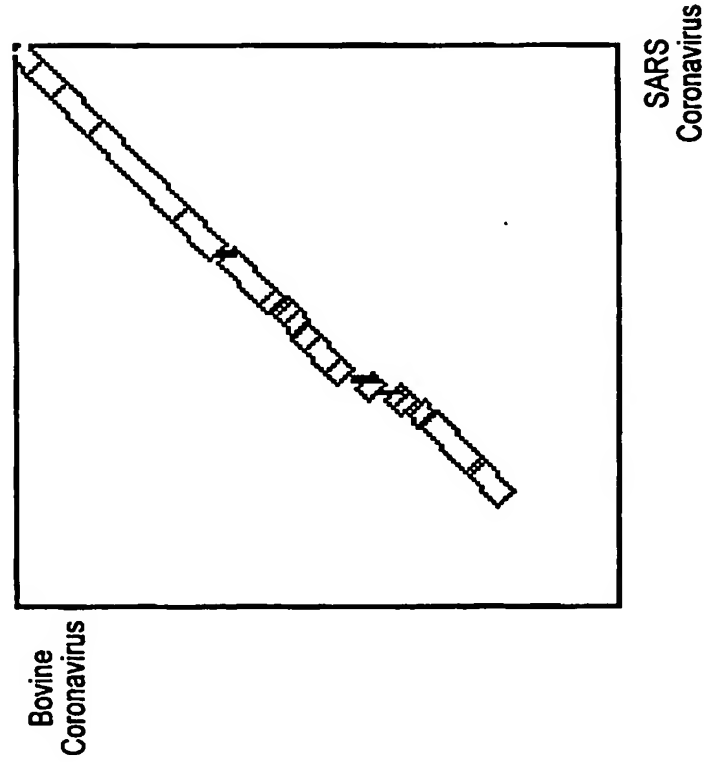


A.A. Sequence Comparison of Spike Proteins
between SARS Coronavirus with Human
Coronavirus OC43



Identities = 349/1122 (30%), Positives = 524/1122 (45%),
Gaps = 148/1122 (13%)

A.A. Sequence Comparison of Spike Protein
between SARS Coronavirus with Bovine
Coronavirus



Identities = 349/1122 (31%), Positives = 524/1122 (46%),
Gaps = 148/1122 (13%)

Figure 2

Figure 1

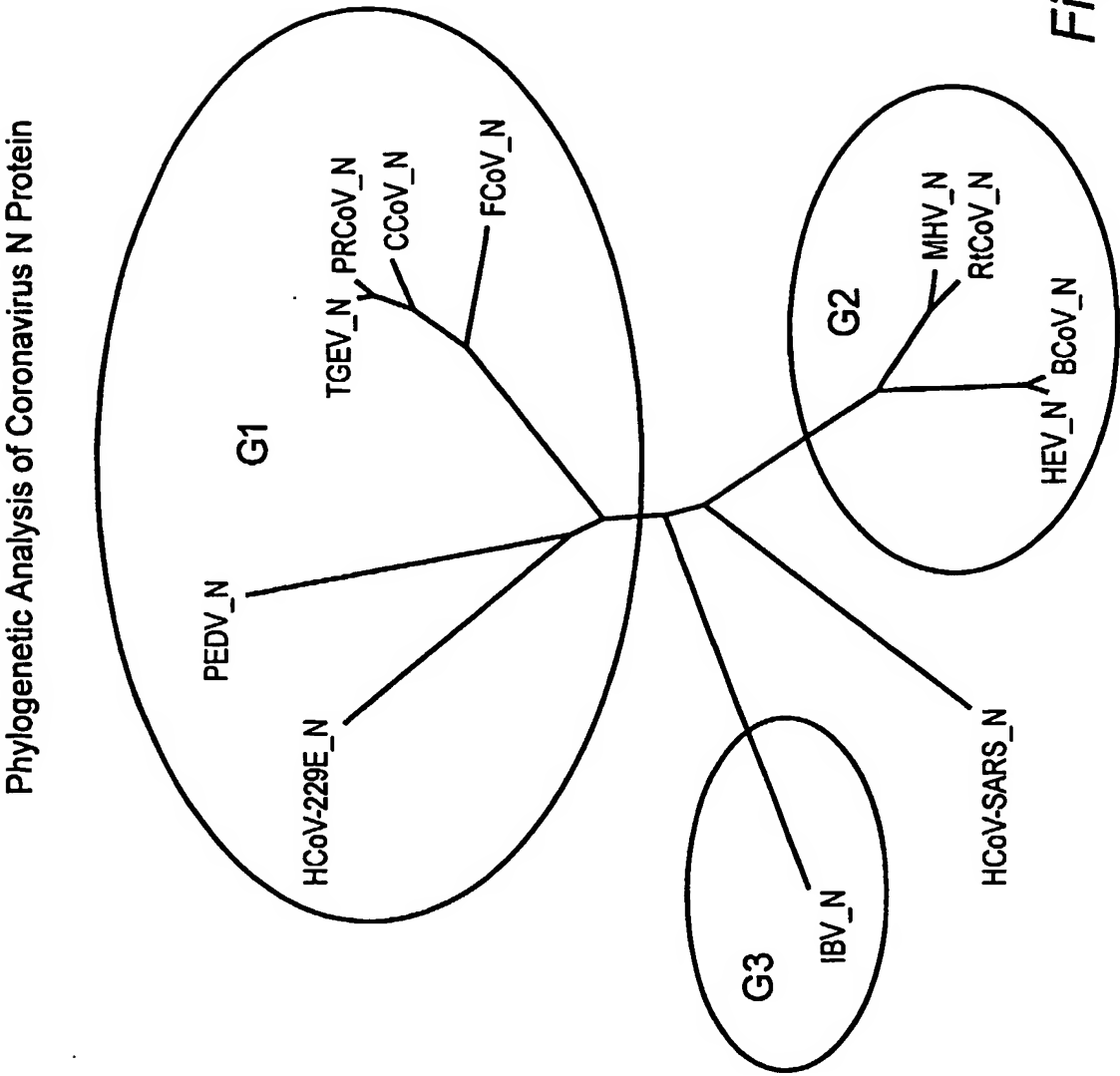


Figure 3

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Phylogenetic Analysis of Coronavirus S Protein

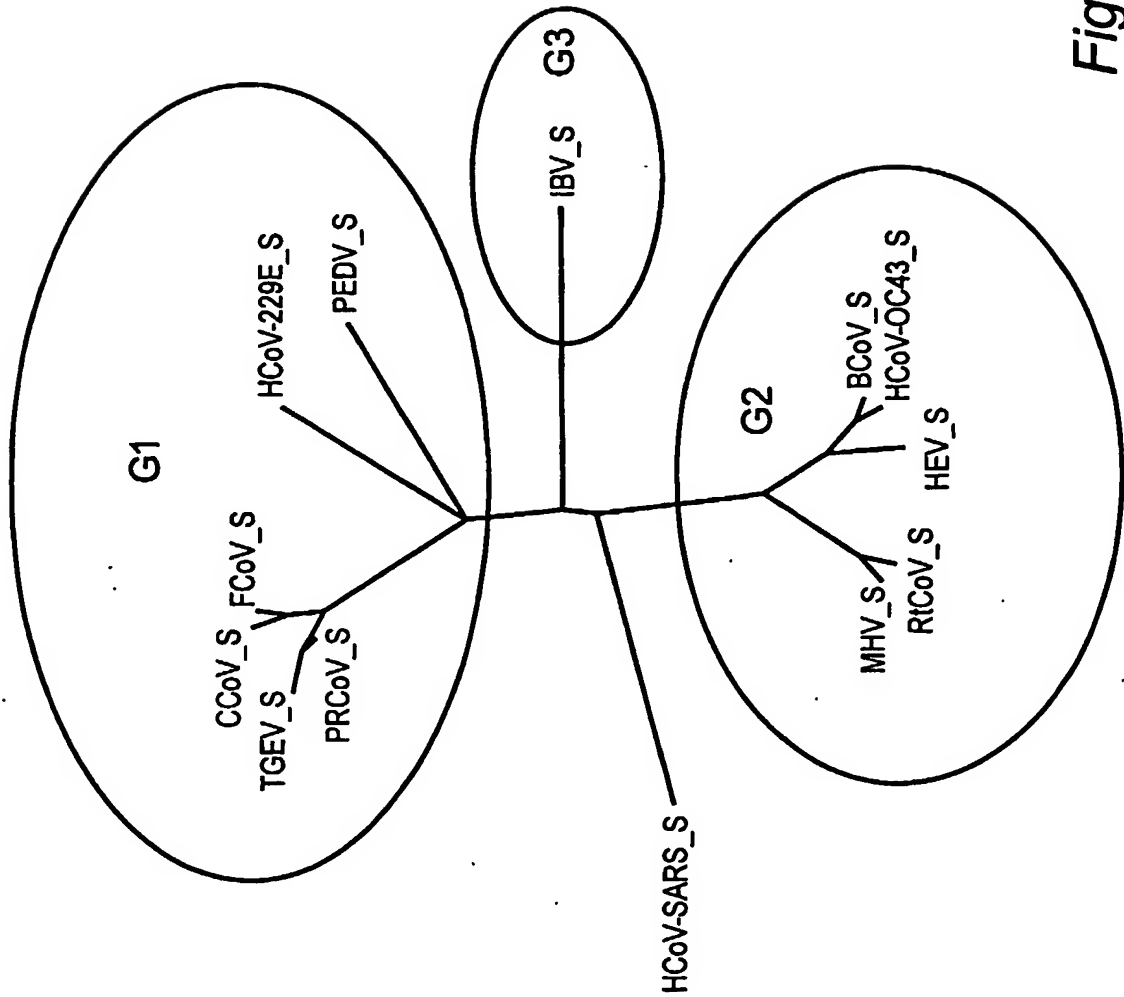


Figure 4

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Phylogenetic Analysis of Coronavirus M Protein

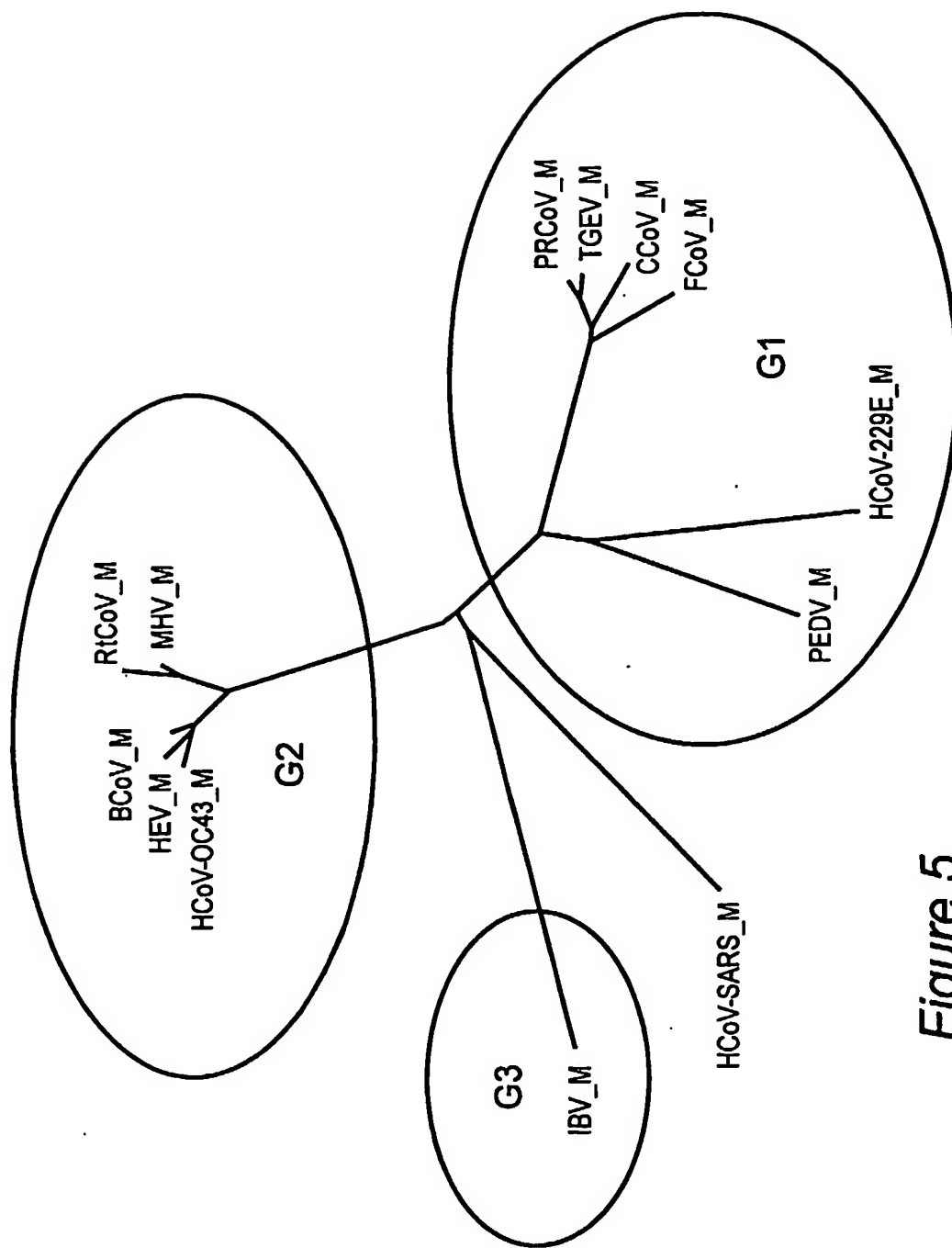
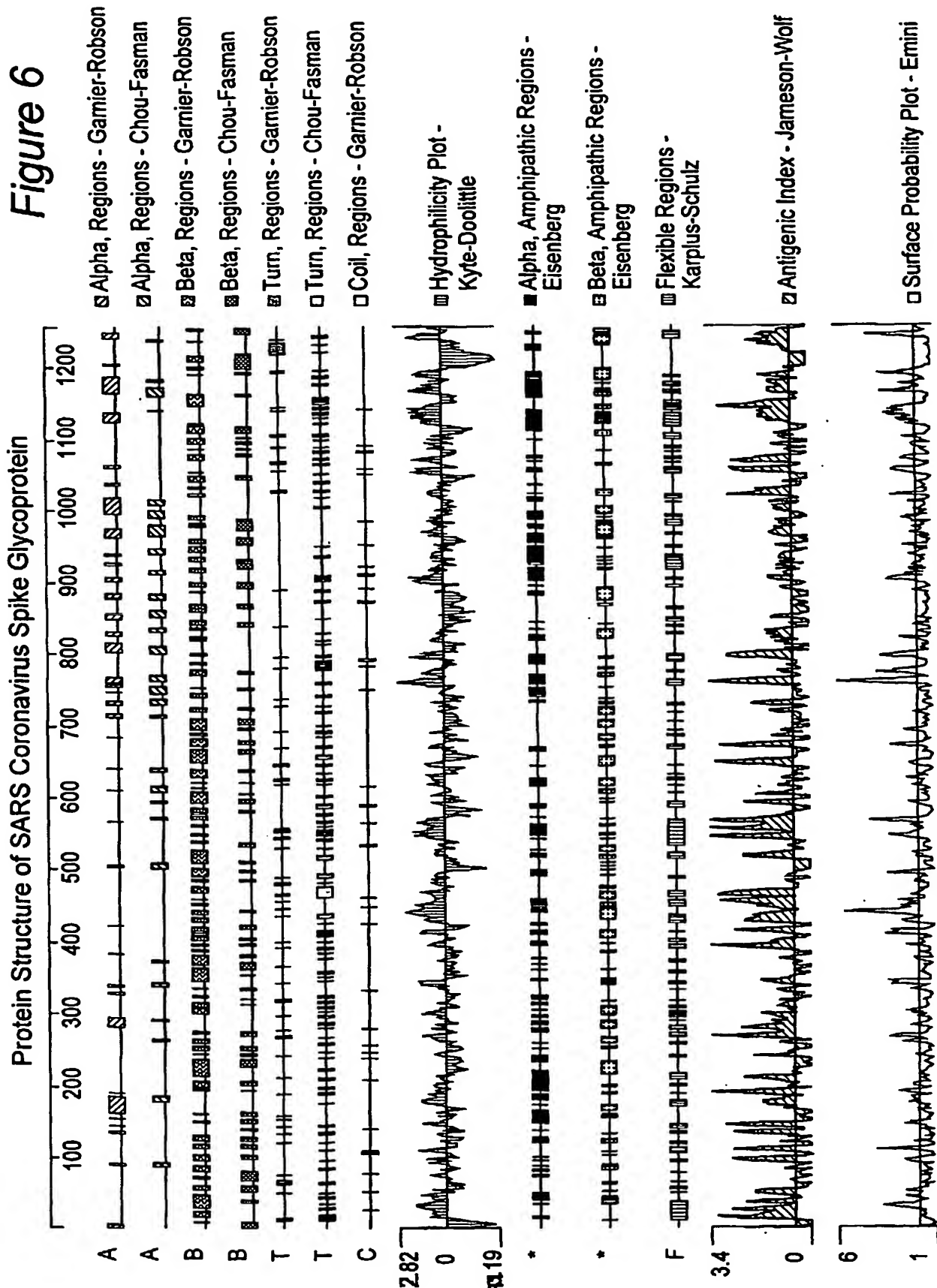


Figure 5

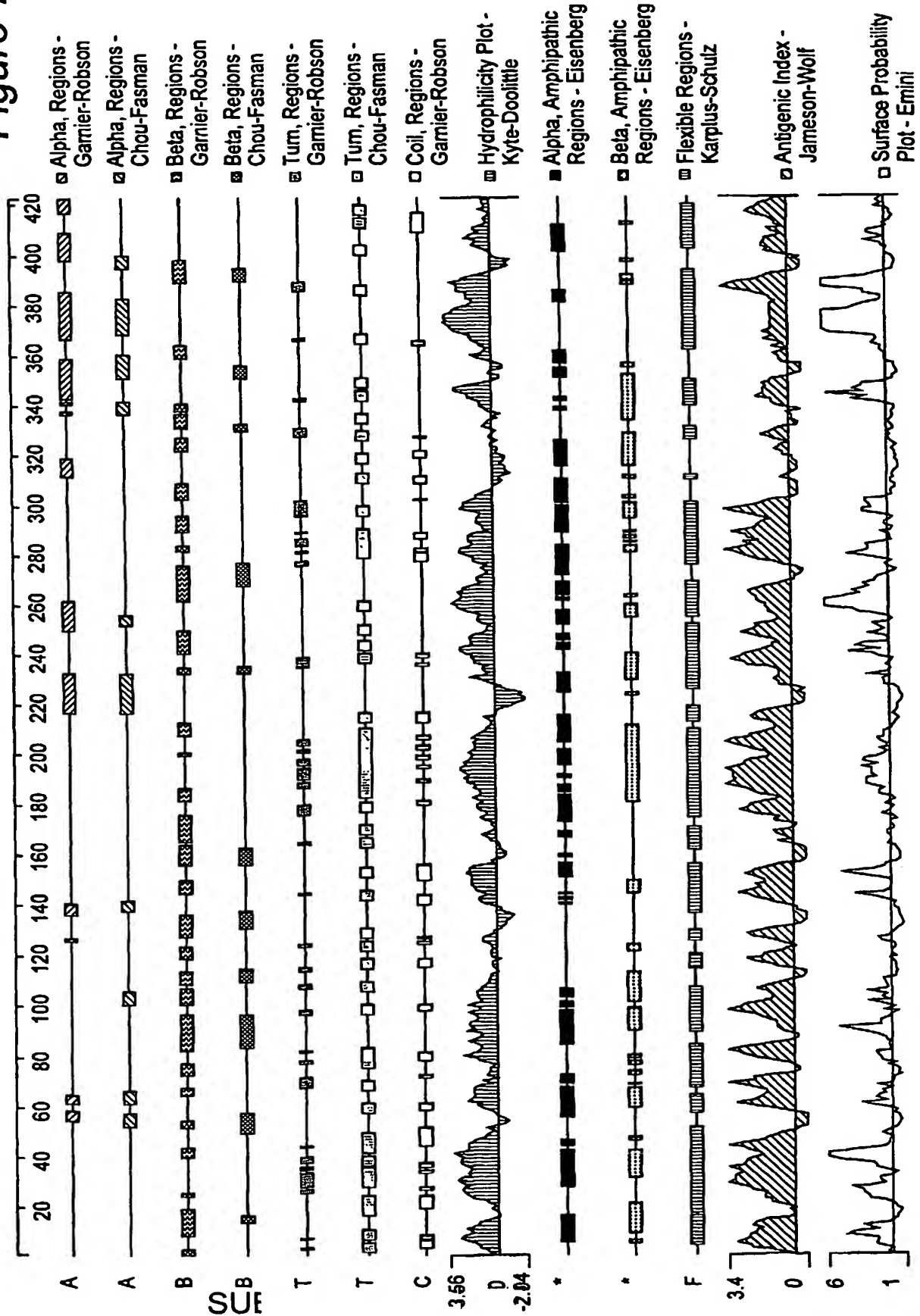
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Figure 7

Protein Structure of SARS Coronavirus NP Protein



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SARS Spike Protein Peptides

Name of peptide	Amino acid sequence	a.a position
DUHVI SA-S1	TTFDDVQAPNTQHTSSMRGVYYPDEIFRSDT	20-51
DUHVI SA-S2	FKDGIYFAATEKSNVVRGWVFGSTMNKSQS	83-113
DUHVI SA-S3	NSTNVIRACNFELCDNPFFAVSKPMGTQTH	119-149
DUHVI SA-S4	DVSEKSGNFKHLREFVFNKDKGFLYVYKGYPIDVVRDLPSG	171-213
DUHVI SA-S5	FSPAQDIWGTSAAYFVGYLKPTTFMLKYDENGITIT	238-273
DUHVI SA-S6	KYDENGITITDAVDCSQNPLAELK	265-287
DUHVI SA-S7	FSPAQDIWGTSAAYFVGYLKPTTFMLKYDENGITIT	288-320
DUHVI SA-S8	FVVGDDVRQIAPGQTGVADIADYNYKL PDDFM	386-417
DUHVI SA-S9	NTRNIDATSTGNYNKYRYLRHGKLRPFERDISN	424-457
DUHVI SA-S10	FSPDGKPCPTPALNCYWPLNDYGYFTTTGIG	460-490
DUHVI SA-S11	PKLSTDLIKNCVNFNGLTGTGVLTPSSKRFG	513-546
DUHVI SA-S12	TPSSKRFPQFQGRDVSDFDTSVRDPKTSE	539-569
DUHVI SA-S13	TNASSEVAVLYQDVNCTDVSTAIHADQLTPAWRIYSTGN	588-626
DUHVI SA-S14	EHVDTSYECDIPIGAGICASYHTVSLRSTSQKSI	640-674
DUHVI SA-S15	EHVDTSYECDIPIGAGICASYHTVSLRSTSQKSI	753-782
DUHVI SA-S16	LKPTKRSFIEDLLFNKVTLADAGFMKQYGECLGINARDL	792-831
DUHVI SA-S17	NQKQIANQFNKAISQIQESLTTTSTALGKLQDVVNQNAQ	901-939
DUHVI SA-S18	SKRVDFCGKGYHLMSPQAAAPHGVVFLHVTYVPSQERNF	1019-1057
DUHVI SA-S19	EGKAYFPREGVVFVNGTTSWFTITQRNFFSP	1066-1094
DUHVI SA-S20	DPLQPELDSFKEELDKYFNKHTSPDVLGDISG	1121-1153
DUHVI SA-S21	QKEIDRLNEVAKNLNESLIDLQELGKYEYQ	1162-1191
DUHVI SA-S22	LTVLPPLLTDDMIAAYTAALVSGTATAGWTFGAGALQIPF	841-882
DUHVI SA-S23	AMQMAYRFGIGVGTQNVLYENQKQIANQFNKAISQIQESL	843-921
DUHVI SA-S24	ELDSFKEELDKYFNKHTSPDVLGDISGINASVV	1127-1161
DUHVI SA-S25	NIQKEIDRLNEVAKNLNESLIDLQELGKYEYIKWPW	1162-1197

Figure 8

SARS NP Protein Peptides

Name of peptide	Amino acid sequence	a.a position
DHVI SA-N1	DSTDNNQNGRNGARPKQRRPQGLPNN	23-49
DHVI SA-N2	GSRGGSQASSRSSRSRGNSTPGSSRGNSPAR	176-210
DHVI SA-N3	KVSGKGQQQQGQTVTKKSAAEASKPRQKRATK	234-267
DHVI SA-N4	GRRGPEQTQGNFGDQDLIRQGTDYKH	276-301
DHVI SA-N5	HIDAYKTFPPTPEPKDKKKTKTDEAQLPQRQKKQ	357-369
DHVI SA-N6	QKKQPTVTLPLPAADMDDFSRQLQNSMSGASADSTQ	387-421

Figure 9

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Coronavirus Spike Protein Among Isolates

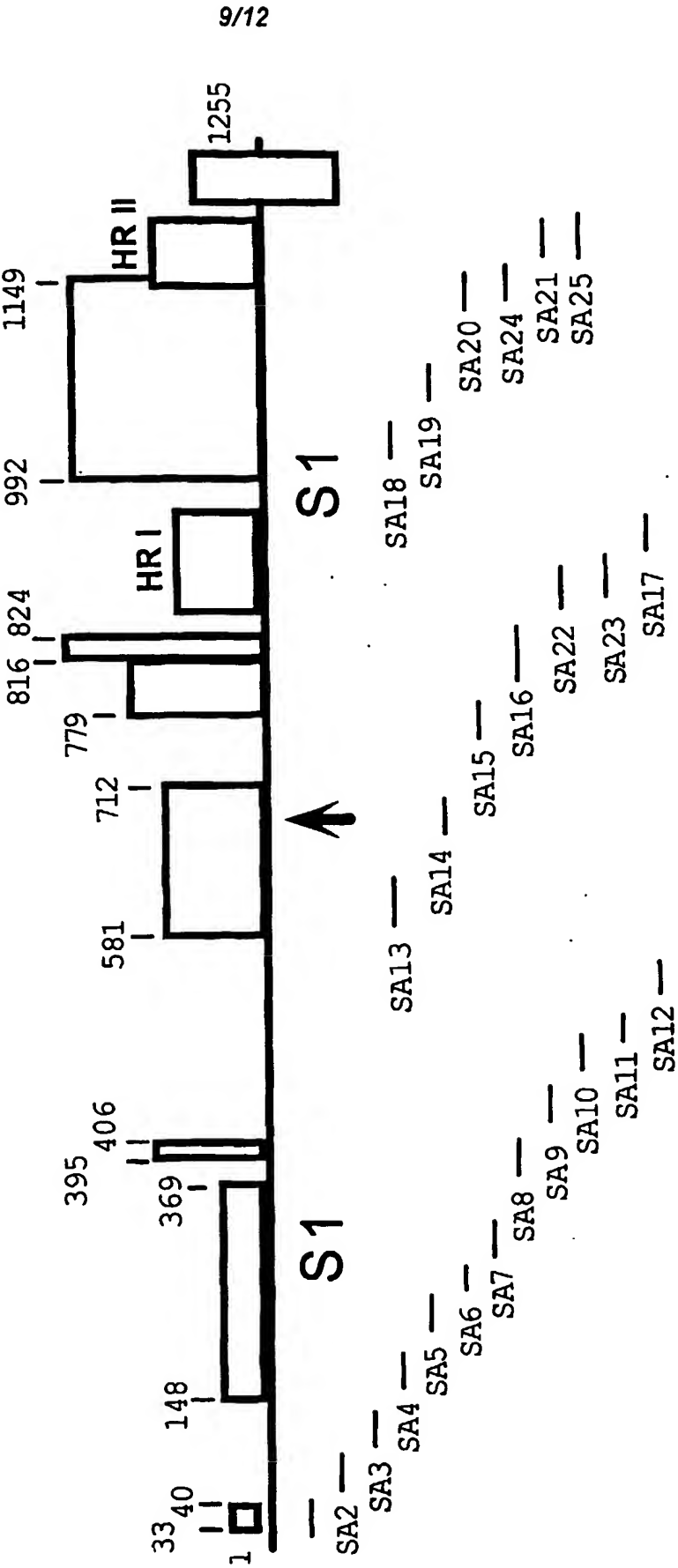
SARS isolate:	0	77	244	311	325	330	344	381	577	778	1163	1255
SARS TOR2		G	I	G	F	N	K	N	A	Y	K	
SARS Urbani (CDC)		G	I	G	F	N	K	N	S	Y	K	
SARS CUHK-W1		D	T	G	F	N	K	N	S	Y	K	
SARS HUK-39849		G	I	G	F	N	K	N	S	Y	K	
SARS BJ01		--		G	--	--	--	N	S	Y	K	
SARS BJ02		D	I	G	L	N	K	N	S	Y	K	
SARS BJ03		--	I	G	F	Y	K	K	S	Y	K	
SARS BJ04		--	I	G	F	N	K	N	--	--	K	
SARS GZ01		--	--	R	F	N	R	N	S	D	E	

-- = sequence not available

Figure 10

Figure 11

Peptide Design Based on Predicated SARS Spike Protein Antigenic Epitopes

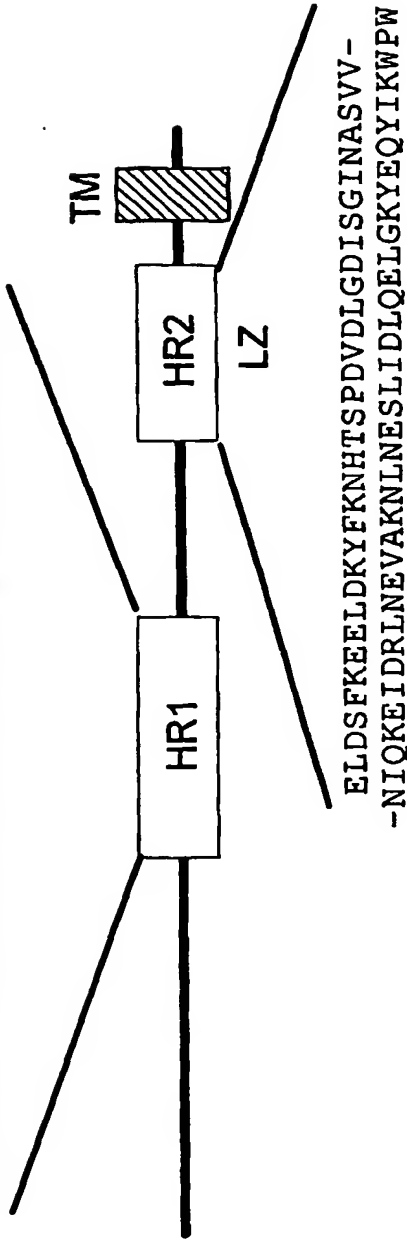


Daniel C et al. J. Virol. 67:1185-1194, 1993; Routledge et al. J. Virol. 65:254-262, 1991; Talbot P.J. et al. J. Virol. 62:3032=3036, 1988.

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HR and LZ Domains in Coronavirus Spike Proteins

AMQMAYRFNGIGVTQNVLYENQKQIANQFNKAISQIQESL-
-LTVLPPLLTDMMIAAYTAALVSGTATAGWTFGAGAAALQIPF



LZ

SARS TOR2 1125 PELDSFKEELDKYFKNHTSPDVLG-DISG

Hu coronavirus 1238 PNLPDFKEELDQWFKNQTLVAPDLSLDY--
Bo coronavirus 1238 PNLHDFKEELDQWFKNQTSVAPDLSLDY--
MHV 1105 PNLPDFKEELDQWFKNQTSIAPDLSLDFEK

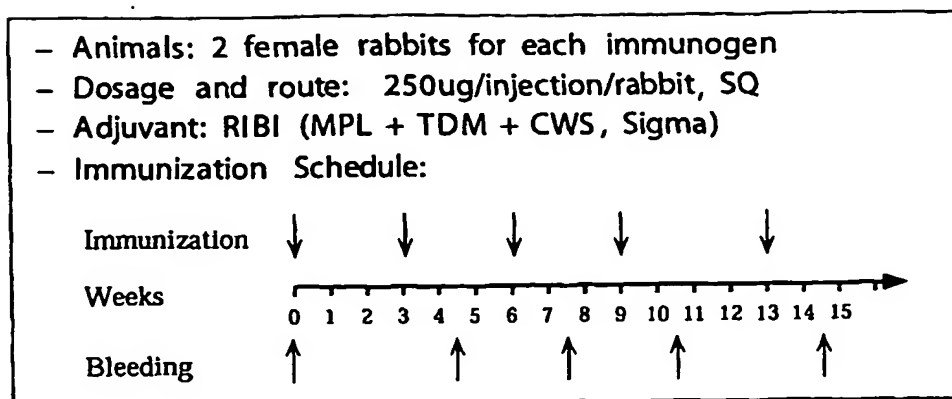
SARS TOR2 1154 I NASVVNIQKEIDRLNEVAKNLNESLIDLQELGKYEQYIKWPW

Hu coronavirus 1266 I NVTFLDLQDEMNRLQEI KVLNQSYI NLKDI GTYEYYVKWPW
Bo coronavirus 1266 I NVTFLDLQDEMNRLQEI KVLNQSYI NLKDI GTYEYYVKWPW
MHV 1135 L NVTFLDLTYEMNRI QDAI KKLNESYI NLKEVGTYEMYVKWPW

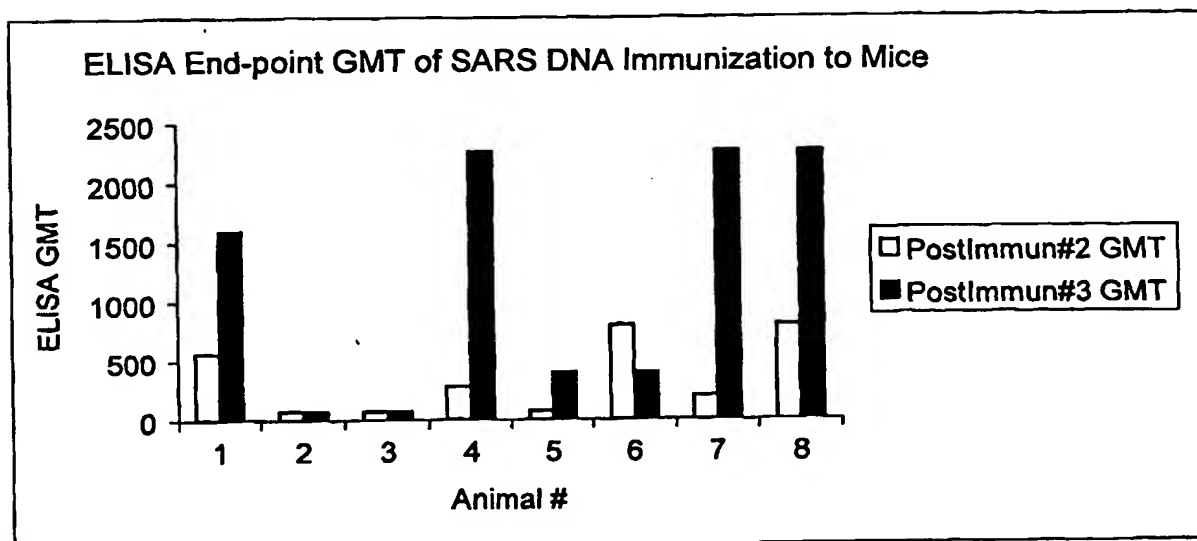
Luo, Z. and Weiss, S.R. In Coronavirus and Arteriviruses, ed by Enjuanes et al. Pp 17-22, 1998

Figure 12

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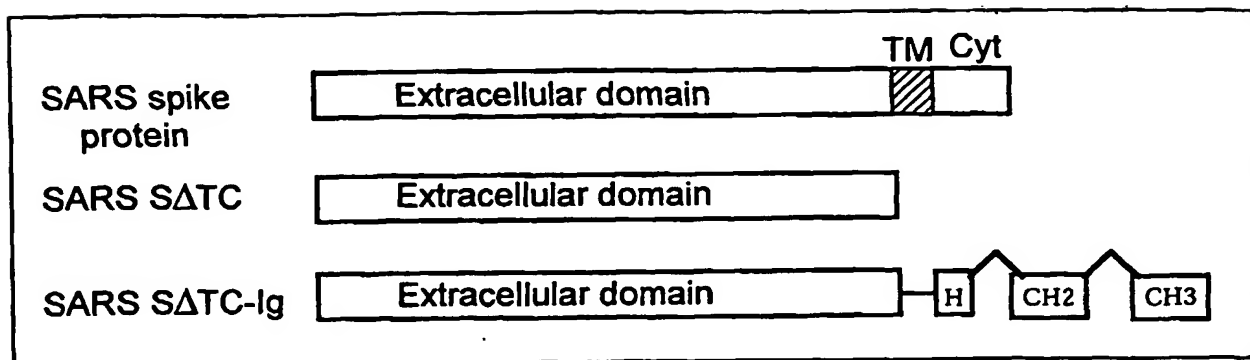
Immunization protocol of rabbits with SARS spike protein peptides

Figure 13

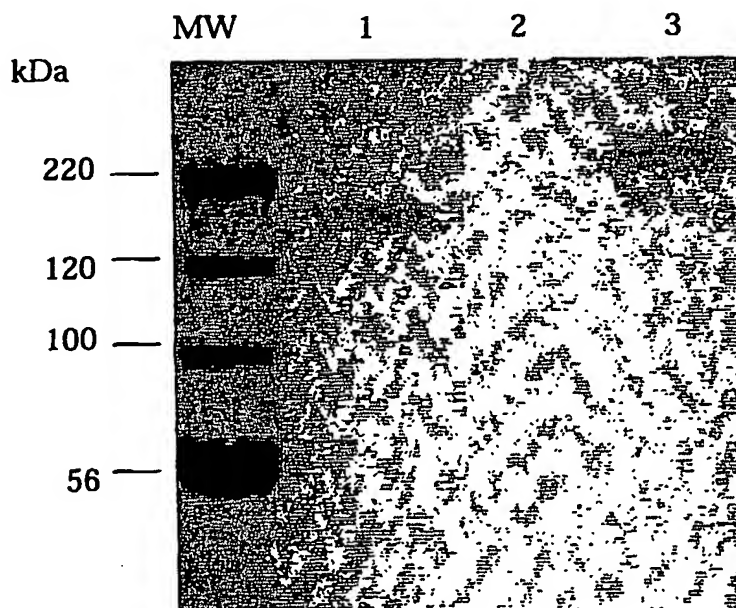
Induction of antibody reacted with recombinant SARS spike protein by immunization with plasmid DNAs express of SARS-spike protein or spike protein-Ig. Serum samples were collected 10 days after immunizations and assayed in ELISA. Show are the end-point ELISA titers against recombinant SARS spike proteins coated on 96-well plate (200ng/well).

Figure 16

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Schematic representation of SARS expression vectors

Figure 14

Western blot analysis of SARS spike proteins. Shown are purified SARS spike protein (lane 1), spike protein-Ig fusion protein (lane 3) and mock transfection supernatant control, which produced in 293 cells by transfection and purified by lectin column were analyzed in Western blot and detected by using immune sera of mouse immunized with DNA vaccine expressing SARS spike protein.

Figure 15